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PRINCIPAL INVESTIGATOR:
Jason D. Weber, Ph.D.

CONTRACTING ORGANIZATION:
Washington University School of Medicine

St. Louis, MO 63110

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14. ABSTRACT
In this second annual review, we have demonstrated that *Arf*-null MMECs contain unique polysome mRNA profiles, up-regulated Drosha levels, and a novel miRNA signature. We have also determined that significant crosstalk exists between the MAPK and mTOR pathways to ultimately regulate ARF mRNA translation in response to oncogenic RasV12 signals. We now understand the translational regulation of NPM. We have identified FBP1 as a key binding protein and repressor of the NPM 3'-UTR. We now have mouse models aimed at understanding the complex functional interactions between ARF, NPM, and DDX5. We have generated two double knockout mouse models that help to clarify the perceived interaction of these proteins. Loss of one allele of *Npm1* or *Ddx5* results in a partial rescue of *Arf* loss with regard to tumor incidence. Taken together, these insightful findings bring us significantly closer to our goal of understanding how signaling pathways impact major tumors suppressors in the control of cell growth.

15. SUBJECT TERMS
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Table of Contents

Page

INTRODUCTION

Cancers result from an inability of a cell to control its own growth. Normally, a cell interprets external and internal signals to create a balanced growth schedule. The main interpreters of these signals within a cell are called ARF and p53, and it falls on the shoulders of these two proteins to maintain normal cell growth. In this sense, both ARF and p53 are tumor suppressors that constantly monitor the growth state of the cell. In mouse and human cancers, loss of the ARF tumor suppressor is second only to mutation of p53, providing critical evidence of ARF's role in both monitoring and preventing the outbreak of cancer cells. A common target of ARF is the NPM/B23 oncogene, an abundant protein of the nucleolus. NPM normally responds to growth factors and, due to its nucleolar localization, is thought to transmit these growth signals to the maturing ribosome machinery. Cells lacking *Arf* exhibit tremendous gains in ribosome production and subsequent protein synthesis. Moreover, the entirety of this growth phenotype is dependent on NPM and p68DDX5 expression in the nucleolus, with loss of either capable of completely reversing the phenotype back to normal. This exciting new finding indicates that ARF is a master regulator of cell growth through its tight control of NPM- or DDX5-directed ribosome production and export. Importantly, we have found NPM overexpressed in nearly 50% of breast carcinomas that we have analyzed, implying that dysregulation of NPM may be a key event in promoting breast cancer development. In effect, tumor cells that require increased protein synthesis might accumulate more NPM or DDX5 in an attempt to increase ribosome output. It is our goal to determine whether NPM directly regulates ribosome maturation to promote breast cancer formation and to establish the importance of ARF in deterring this effect. We propose to now determine the complex roles of ARF, DDX5, and NPM in the nucleolus of breast epithelial cells and how they impact both ribosome biogenesis and cell growth to prevent and/or promote tumorigenesis.

This work has tremendous clinical implications as *Arf* (9p21) and *p68Ddx5* (17q24) reside on loci that are either deleted or amplified in ER+ resistant breast tumors, respectively. This fact makes our basic science on this interesting growth network directly applicable to the breast cancer phenotype/genotype.

BODY

As stated in the approved Statement of Work, we focused our energies on the tasks planned for Months 13-24. These included experiments outlined in Tasks 1 and 2. In the last two months of this fiscal year (January and February 2010) we have initiated experiments in task 3. In this second Annual Progress Report, we detail the progress and results from these studies.

Task 1. Determine the role of ARF in suppressing breast tumor formation (Months 1-30):

d. Generate and validate polysome microarray profiles for wild-type and *Arf*-/- MECs (Months 10-30).

During the second year of this grant, we have focused our efforts more broadly on completing the experiments outlined in Tasks 1 and 2. For Task 1, this included the challenging job of generating polysome arrays derived from wild-type and Arf-deficient cells. In this endeavor, we have been extremely successful. We isolated 2 μg of polysome-associated mRNA from fractions harvested by gradient centrifugation and constant UV monitoring (**Figure 1**). Using this standard technique, it is clear

that cells lacking *Arf* contain greater numbers of polysomes actively translating mRNAs (**Figure 1**, compare red and blue lines at the far right end of the sucrose gradient).

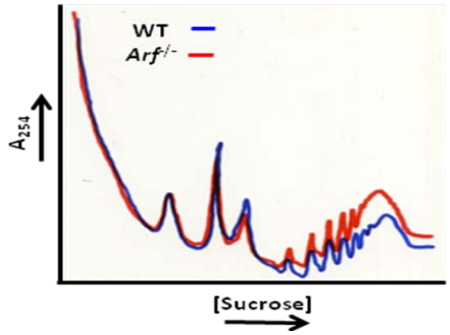

Figure 1. Equal numbers of wild-type (WT) and *Arf*-/- MMECs ($3x10^6$) were isolated and incubated with 50 μg/ml cycloheximide to freeze ribosomes on mRNA. Cells were lysed and separated on continuous sucrose gradients by ultracentrifugation. Fractions were isolated using ISCO constant UV monitoring and plotted as absorbance (254 nm) versus gradient position. The peaks from left to right are 40S, 60S, 80S and polysomes. (Task 1d)

We next isolated mRNA from sucrose gradient fractions taken at 2 ml intervals. Thus, each fraction contained approximately 2 μg of total RNA (mRNA + rRNA). Isolated mRNA was amplified and labeled for microarray analysis. We utilized the mouse 20k gene array from Illumina. We also performed this analysis on identical samples of total mRNA. This allowed us to functionally analyze both the transcriptome (total mRNA) and translatome (polysome mRNA) from wild-type and *Arf*-/- cells. As shown in **Figure 2**, WT and *Arf*-/- total and polysomes did not cluster together. Rather, replicates from each sample clustered nicely which allowed us to perform broad statistical analyses on each sample. From this analysis, we have begun to identify each mRNA whose rate of translation is distinct from its rate of transcription. Using this parameter, we will rank mRNAs based on either increased or decreased translation in the absence of *Arf* in the coming year (months 24-36).

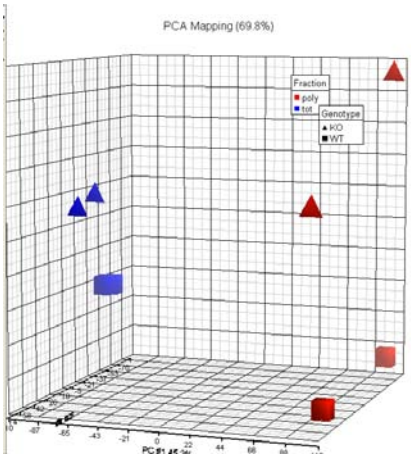

Figure 2. mRNA isolated from polysomes of wild-type (WT) and *Arf*-/- MMECs ($3x10^6$) was hybridized to Illumina bead chips containing mouse mRNA probes spanning over 20,000 genes. Total RNA was also isolated to compare transcriptome to translatome. WT mRNA is depicted as squares and *Arf*-/- as triangles. Total RNA is in blue and polysome RNA is in red. (Task 1d)

e. Determine the influence of ARF on Drosha-mediated RNA translation (Months 11-16).

One hypothesis that could explain the differences in translation we observed in the absence of *Arf* could be a dysregulation of microRNA biogenesis. To assess this possibility, we first investigated the expression levels of Drosha, a key component of primary microRNA processing in the nucleoplasm. Wild-type and *Arf*-/- MMECs were passaged every three days and harvested for western blot analysis using antibodies recognizing mouse Drosha. We found that indeed Drosha protein expression increased

in the absence of *Arf* and continued to increase over 20 passages in culture (**Figure 3**). In the next year, we will determine the mechanism behind this unique finding.

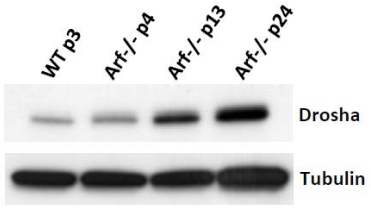

Figure 3. Primary MMECs derived from wild-type (WT) or *Arf*-/- mice were grown in matrigel and passaged every three days and harvested at the indicated passage numbers. Cells were lysed, proteins separated by SDS-PAGE and immunoblotted with antibodies recognizing mouse Drosha and tubulin. (Task 1e)

Having shown that Drosha levels are elevated in the absence of *Arf*, we next sought to determine whether this alteration could also affect the levels of miRNAs produced in the absence of *Arf*. With assistance from the Genome Sequencing Center at Washington University, we were able to perform deep sequencing of mouse miRNAs isolated in WT and *Arf*-/- MEFs. We were unable to produce enough MMECs to perform our initial analysis. However, we plan to perform the identical experiment in MMECs in the next year. Our deep sequencing showed a clear dysregulation of miRNA synthesis in the absence of *Arf* that was only more pronounced as cell were passaged in vitro (**Figure 4**). This result suggests that miRNAs deregulated in the absence of *Arf* might have a more direct impact on the translatome than previously thought. In the next year, we will begin to characterize the effects of individual miRNAs identified in our deep sequencing and parse their potential effects on cell growth and transformation in the absence of *Arf*.

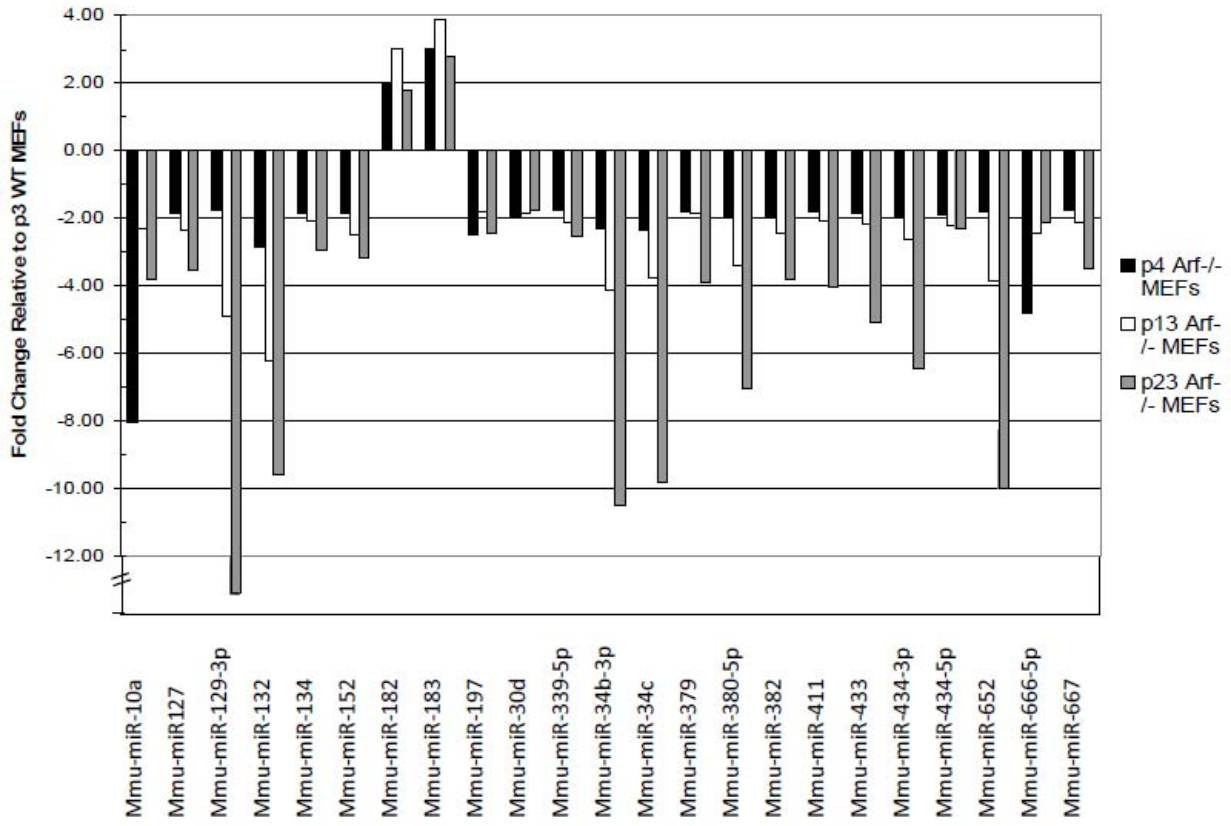

Figure 4. Primary *Arf*-/- mouse embryonic fibroblasts were harvested at the indicated passages and microRNA was isolated. MicroRNAs were subjected to deep sequencing and internally controlled for by U6 RNA levels. Values for fold-change are representative of three independent experimental sequencing runs. (Task 1e)

f. Identify the signaling pathway(s) responsible for enhanced ARF mRNA translation (Months 12-24).

We had previously shown that the ARF tumor suppressor was regulated post-transcriptionally by the mTOR pathway. In order to establish the mechanism behind this protein induction, we investigated the ability of the more classical MAPK pathway to also activate ARF protein expression. Primary WT MMECs infected with retroviruses encoding either constitutively activate RasV12 or MEK-Q56P were harvested and immunoblotted against MAPK and mTOR pathway proteins. Infection with RasV12 led to tremendous increases in ARF protein expression (**Figure 5**, lane 2). However, ARF was also significantly induced by constitutively activated MEK (**Figure 5**, lane 3), although not to the extent of RasV12. This suggests that MAPK may in fact be regulating ARF expression independent of mTOR. However, the result is compounded by the fact that we also observed equal S6 phosphorylation with MEK-Q56P, suggesting that there may be some level of crosstalk between MAPK and mTOR pathways. In the next year, we will determine the mechanism behind this apparent crosstalk and determine if it is the reason for ARF induction in the absence of classical mTOR signals (no phospho-AKT in lane 3).

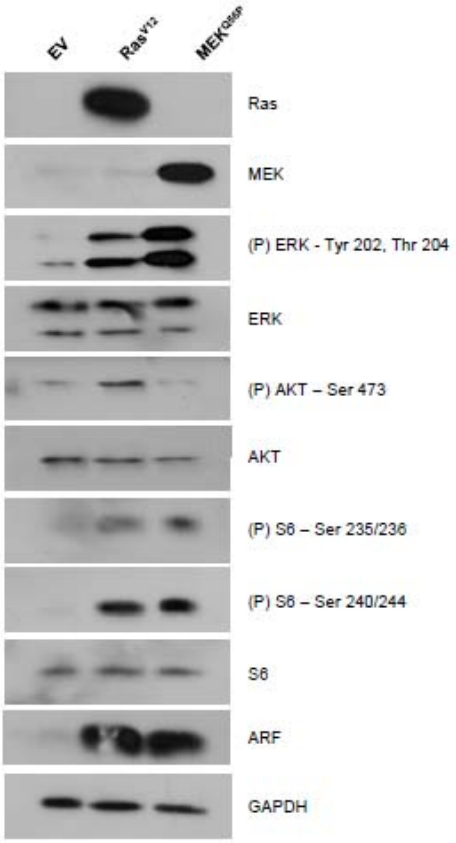

Figure 5. Primary wild-type MMECs were infected with retroviruses encoding oncogenic RasV12 or activated MEKQ56P and selected with puromycin for 48 hours. Following selection, cells were harvested and subjected to SDS-PAGE and immunoblot analysis with antibodies recognizing each of the indicated proteins (labeled on the right). GAPDH served as an internal protein expression control. (Task 1f)

g. Determine the mechanism for ARF translation (Months 12-30).

To determine the mechanism for ARF protein induction following activated mTOR signals, we utilized *Tsc*fl/fl MMECs treated with control LacZ or Cre-adenoviruses. Using this method, we had previously shown that mTOR is hyperactivated and that ARF protein is induced. We now show that in the presence or absence of *Tsc*, ARF mRNA is equally stable (**Figure 6**), suggesting that increased ARF mRNA stability is

not the mechanism for the apparent increases seen in ARF protein in the absence of *Tsc*. Furthermore, we went on to show that the ARF protein is equally labile in the presence or absence of *Tsc* (**Figure7**), again indicating that stability is not the reason for increased ARF protein expression in the presence of hyperactivated mTOR signals.

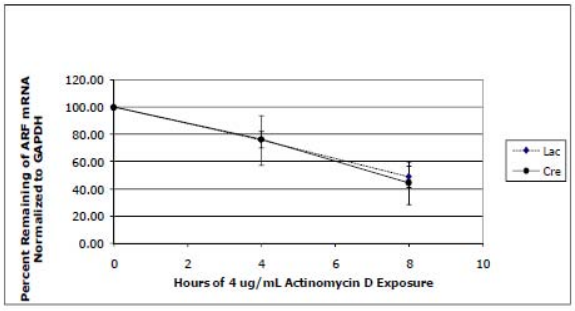

Figure 6. *Tsc*fl/fl MMECs were infected with adenoviral LacZ or Cre and harvested 10 days post-infection following indicated pulses of actinomycin D. ARF mRNA was analyzed by qRT-PCR using probes unique to ARF exon 1β. ARF mRNA stability was plotted against GAPDH control to determine half-life of 7 hours. (Task 1g)

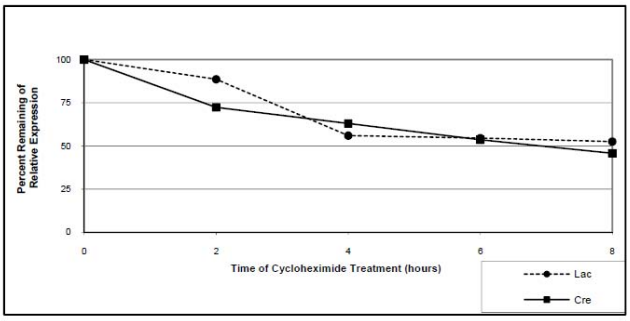

Figure 7. *Tsc*fl/fl MMECs were infected with adenoviral LacZ (dashed line) or Cre (solid line) and harvested 10 days post-infection following indicated pulses of cycloheximide. ARF protein was analyzed by immunoblotting with antibodies recognizing mouse ARF. ARF protein stability was plotted against tubulin control to determine half-life of 6 hours. (Task 1g)

In the next year, we will complete Task 1 in its entirety and plan on submitting the data generated to a peer-reviewed scientific journal. This will complete our studies which strived to determine mechanism behind enhanced ARF translation in the absence of *Tsc1* using genetically-modified MMECs.

Task 2. Examine the mechanism behind NPM's ability to promote ribosome biogenesis and cell growth in breast epithelial cells (Months 1-36):

c. Validate the responsiveness of a novel 5'-3' NPM-TOP luciferase reporter construct to in vitro mTOR signals (Months 12-36).

Recognition and binding of elements within the 5' and 3' UTRs of mRNAs by regulatory proteins is a common mechanism underlying selective mRNA translational control (1). Indeed, previous reports have indicated that various mRNAs are subject to such regulation (2-7). To determine whether a comparable mechanism may be responsible for the translational regulation of NPM, we first identified the 5' UTR sequence of the NPM transcript by rapid amplification of cDNA ends (RACE) (GenBank accession number GU214027). Like the human NPM 5' UTR (8), RACE revealed that the murine NPM 5' UTR contains a canonical terminal oligopyrimidine tract (TOP) also contained in the 5' UTRs of transcripts encoding ribosomal proteins, elongation factors, and other components of the translational machinery (9, 10). We attained the complete NPM and GAPDH 3' UTR sequences from GenBank (accession numbers

BC054755.1 and NM_008084.2, respectively).

We sought to evaluate whether the NPM 5' and 3' UTRs were sufficient to modulate translation of another ORF in a manner equivalent to translational regulation of the NPM ORF. Specifically, we wanted to determine whether fusion of the NPM 5' and 3' UTRs to a firefly *luciferase* (F*luc*) ORF rendered Fluc expression sensitive to rapamycin. To test this, *Tsc1*$^{-/-}$*p53*$^{-/-}$ MEFs were transduced with plasmids encoding NPM 5' and 3' UTR-flanked F*luc*. Although NPM 5'-luc-NPM 3' protein activity increased over the duration of serum stimulation, this induction was greatly attenuated in the presence of rapamycin compared to vehicle (**Figure 8*A***). These data indicate that NPM 5'-luc-NPM 3' activity is driven by changes in translation rather than transcription. To examine whether the rapamycin-induced reduction of NPM 5'-luc-NPM 3' activity was specific for an mTOR-regulated mRNA, *Tsc1*$^{-/-}$*p53*$^{-/-}$ MEFs were transduced with plasmids encoding GAPDH 5' and 3' UTR-flanked F*luc*. Notably, rapamycin failed to affect GAPDH 5'-luc-GAPDH 3' activity at any time point evaluated (**Figure 8*B***).

To examine the independent roles of each NPM UTR as potential targets of regulation, we generated chimeric reporters by fusing the NPM 5' UTR and the GAPDH 3' UTR or the GAPDH 5' UTR and the NPM 3' UTR to the respective ends of the F*luc* ORF. Surprisingly, NPM 5'-luc-GAPDH 3' activity resembled GAPDH 5'-luc-GAPDH 3' activity, with rapamycin having no effect at any time point measured (**Figure 8*C***). GAPDH 5'-luc-NPM 3' activity, however, demonstrated rapamycin sensitivity similar to that observed with NPM 5'-luc-NPM 3' activity (**Figure 8*D***). Collectively, these data suggest that sequences within the NPM 3' UTR, but not in the NPM 5' UTR, mediate regulation of NPM mRNA translation, as the NPM 3' UTR alone was sufficient to render the F*luc* ORF rapamycin-sensitive. Given that rapamycin sensitivity of 5' TOP mRNAs ranges from resistance to marked repression (11), these data are in accordance with the poorly understood role of the 5' TOP. Our findings are consistent with reports highlighting the paucity of regulatory protein-RNA interactions in the 5' UTR, but the abundance of examples for 3' UTR-protein regulation (12).

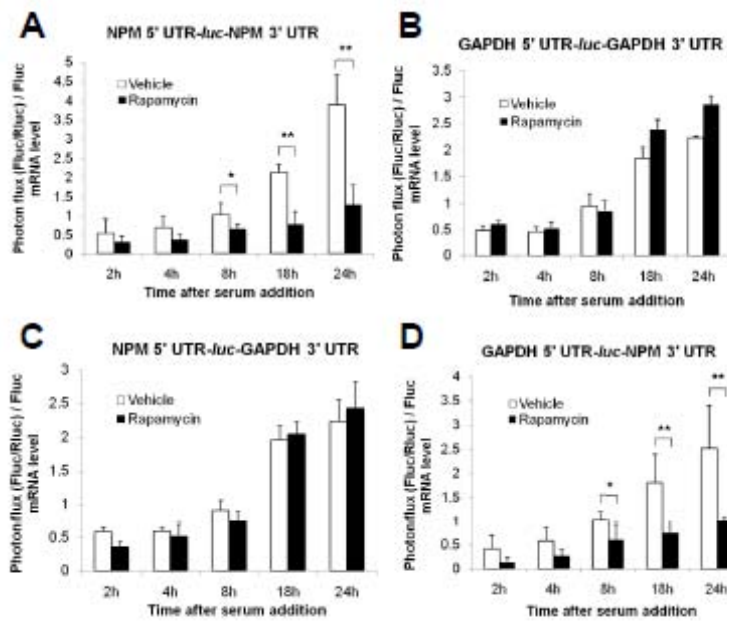

Figure 8. (*A-D*) *Tsc1*$^{-/-}$*p53*$^{-/-}$ MEFs were transfected with plasmids depicted in S2*B*. Cells were serum starved and then incubated with 10% serum in the presence or absence of rapamycin for the indicated durations. Plasmid expressing CMV-driven *Renilla* luciferase (Rluc) was used as an internal control for transfection efficiency. Photon flux was calculated by normalizing firefly *luciferase* (Fluc) activity to Rluc activity. Levels of Fluc mRNA at each time point were measured by qRT-PCR from total RNA isolated from transfected MEFs. Shown is photon flux normalized to Fluc mRNA levels. Data are mean ± s.d. of quadruplicate samples per condition from three independent experiments (* $P < 0.05$, ** $P < 0.005$, Student's *t*-test). (*A*) Rapamycin reduces NPM 5' UTR-Fluc-NPM 3' UTR activity. (*B*) Activity of GAPDH 5' UTR-Fluc-GAPDH 3' UTR is unchanged upon treatment with rapamycin. (*C*) Rapamycin has no effect on NPM 5' UTR-Fluc-GAPDH 3' UTR activity. (*D*) Activity of GAPDH 5' UTR-Fluc-NPM 3' UTR is abrogated upon rapamycin treatment. (Task 2c)

We are now poised to test these constructs in MMECs to determine whether this regulation is more general.

d. Identify proteins that bind to the 5' and 3' UTR of NPM mRNA to regulate its translation (Months 10-36).

Although reporter assay data (**Figure 8*A-D***) indicated that only the NPM 3' UTR is important for modulation of the NPM mRNA, we undertook an unbiased approach to screen for putative regulatory binding proteins of the NPM 5' and 3' UTRs. We utilized an RNA pull-down assay coupled to mass spectrometry to identify proteins that bind the NPM 5' or 3' UTR. Whole cell lysates prepared from *Tsc1*$^{-/-}$*p53*$^{-/-}$ MEFs treated with vehicle or rapamycin were incubated with biotinylated NPM 5' UTR or 3' UTR RNA. Several proteins were found to preferentially interact with the NPM 3' UTR, but none appeared to bind exclusively to the NPM 5' UTR, consistent with reporter assay findings (**Figure 9**, arrows). We next employed mass spectrometry to identify putative NPM 3' UTR binding proteins and confirmed their identities as FBP1, FBP2 (also known as KHSRP or KSRP), and heterogeneous nuclear ribonucleoprotein (hnRNP) A/B. Since the A/B subfamily of hnRNPs refers to several promiscuous, multifunctional RNA binding proteins (13), subsequent experiments focused on evaluating the roles of FBPs in NPM translational regulation.

The FBP family is most noted for its transcriptional regulation of c-*myc* (14, 15); however, the FBPs have also been reported to bind several RNAs, though in *in-vitro* studies only (16). To evaluate FBP

binding specificity, we incubated biotinylated GAPDH 5' UTR, GAPDH 3' UTR, NPM 5' UTR, or NPM 3' UTR RNA with whole cell lysates from *Tsc1*$^{-/-}$*p53*$^{-/-}$ MEFs treated with vehicle or rapamycin. FBP1 and FBP2 were visualized by Western blot analysis of UTR-precipitated samples (**Figure 9**). Although FBP3 was not identified by mass spectrometry, we also analyzed it by immunoblot assay, as it is a member of the highly related FBP protein family. Consistent with analyses from mass spectrometry, however, FBP3 was undetectable. FBP1 was precipitated exclusively by the NPM 3' UTR (**Figure 10A**). FBP2 was precipitated predominantly by the NPM 3' UTR, but also by the GAPDH 3' UTR and the NPM 5' UTR in vehicle-treated cells (**Figure 10A**). The more promiscuous RNA binding by FBP2 is in agreement with previous reports implicating FBP2 in RNA editing, RNA trafficking, RNA stabilization, and RNA decay (17-23).

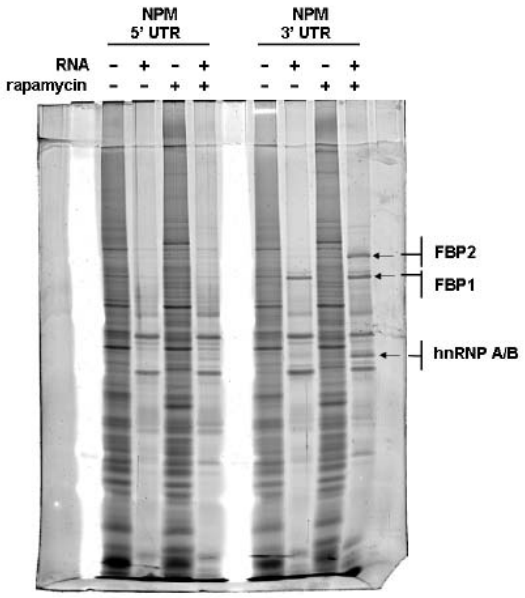

Figure 9. Identification of NPM 3' UTR binding proteins. Lanes indicated as RNA (–) represent samples pre-cleared with streptavidin sepharose. Arrows indicate proteins selected as putative regulatory binding proteins of the NPM 3' UTR, and identified proteins are shown. (Task 2d)

We next sought to verify the interaction of FBP1 with endogenous NPM mRNAs. FBP1 was immunoprecipitated from whole cell extracts prepared from *Tsc1*$^{-/-}$*p53*$^{-/-}$ MEFs treated with vehicle or rapamycin (**Figure 10B**, top). Total RNA was isolated from FBP1 immunoprecipitates, and bound NPM mRNA was measured by qRT-PCR. Significantly higher numbers of NPM transcripts were associated with FBP1 in rapamycin-treated cells versus vehicle-treated cells (**Figure 10B**, bottom). Moreover, FBP1 protein expression dramatically increased upon rapamycin treatment (**Figure 10C**), suggesting that the enhanced number of NPM transcripts bound by FBP1 in the presence of rapamycin was a result of elevated FBP1 expression. We have already begun to move our analysis into MMECs and identified numerous human ER+ breast tumors that harbour significant loss of FBP1 expression.

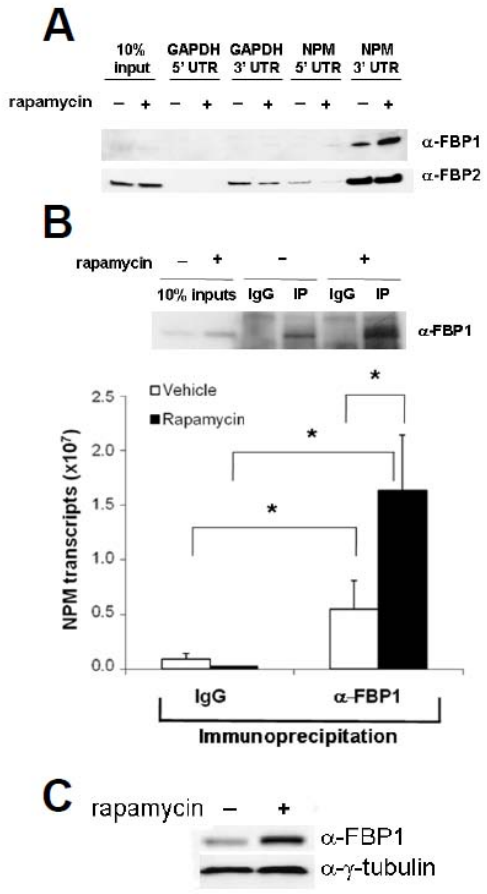

Figure 10. (*A*) FBP1 specifically interacts with the NPM 3' UTR. (*B*) Endogenous NPM mRNAs preferentially bind FBP1 in rapamycin-treated cells. FBP1 was immunoprecipitated (IP) from vehicle-treated (–) or rapamycin-treated (+) *Tsc1*$^{-/-}$*p53*$^{-/-}$ MEF lysates with anti-FBP1 antibody. Non-immune goat serum (IgG) was used as a control. NPM mRNA from immunoprecipitates was measured by qRT-PCR. Data are mean ± s.d. of triplicate samples from three independent experiments (* $P < 0.05$, Student's *t*-test). (*C*) Rapamycin results in increased FBP1 protein expression. (Task 2d)

Task 3. Establish the oncogenic potential of the p68DDX5 RNA helicase (Months 24-48):

b. Determine whether NPM and p68 are phenocopies of one another (Months 24-36).

We had previously shown that ARF regulated both the NPM and p68DDX5 proto-oncogenes independent of p53. If NPM and p68 are unable to be correctly regulated in the absence of ARF, deregulated cell growth ensues. We hypothesized that the levels of NPM and p68 might contribute to this phenotype in vivo. Specifically, unregulated NPM and p68 might promote cell growth in the absence of *Arf* and that by lowering the levels of either ARF target, cell growth might be restored to normal levels. Thus, we crossed *Arf*-/- mice with *p68*+/- and *Npm*1+/- mice obtained through separate funding sources (NIH GM066032 and NIH CA120436). We have followed these double knockouts for over one year now. As shown in **Figure 11**, loss of one copy of *Npm1* or *p68Ddx5* partially rescues the tumor incidence rate of the *Arf*-/- background. We know our animal numbers for the double knockouts are low (3 and 8 mice, respectively). We have three *Arf-/-p68*+/- mice that are still alive after 75 weeks. We also have additional mice (12 and 10, respectively) for each genotype that we are currently following

at the 12 week mark. Win the next year, we will complete the mouse phenotype studies and begin to focus our attention on the in vitro characterization of the MMECs isolated from these mouse cohorts.

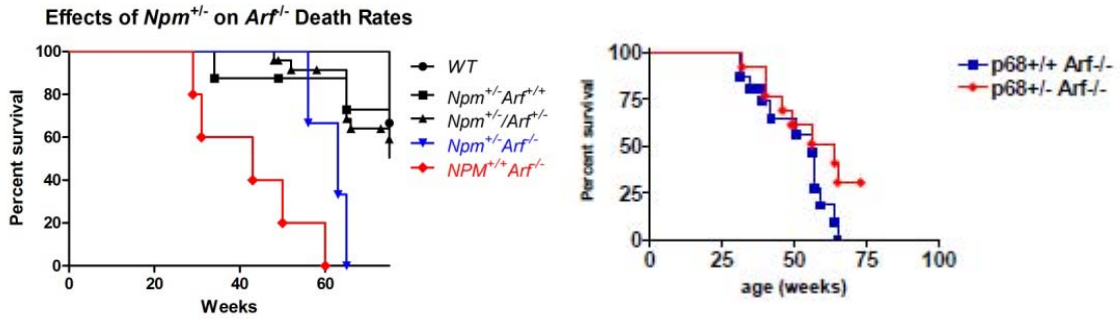

Figure 11. *Arf*-/- mice were crossed into *Npm*+/- or *p68*+/- mice to generate *Arf-/-Npm*+/- or *Arf-/-p68*+/- mice. Each mouse was monitored for tumor formation and scored based on pathology at time of death. Percent survival is calculated based on tumor free survival at time of death. (Task 3b)

KEY RESEARCH ACCOMPLISHMENTS

- *Arf*-null mouse mammary epithelial cells (MMECs) contain unique polysome mRNA profiles (Task 1d)
- Drosha protein expression is elevated in the absence of *Arf* (Task 1e)
- *Arf*-null MECs have a distinct miRNA profile (Task 1e)
- MAPK and mTOR pathways converge to regulate ARF protein expression (Task 1f)
- MAPK cross-talks with the mTOR pathway to regulate ARF (Task 1f)
- ARF mRNA is not transcribed or stabilized more in the presence of RasV12 (Task 1g)
- The 3'-UTR of NPM imparts rapamycin sensitivity (Task 2c)
- The 5' and 3' UTRs of NPM work together to regulate NPM translation in response to growth stimuli (Task 2c)
- FBP1 interacts with the 3'-UTR of NPM to halt NPM translation (Task 2d)
- *Npm1*+/- and *Ddx5*+/- genotypes partially rescue the *Arf*-/- mouse tumor phenotype (Task 3b).

REPORTABLE OUTCOMES

Manuscripts: Brady, S.N., Maggi, L.B., Winkeler, C.L., Pelletier, C.L. and **Weber, J.D.** (2009). Nucleophosmin protein expression level, but not threonine 198 phosphorylation, is essential in growth and proliferation. *Oncogene,* 28:3209-3220.

Abstracts/Presentations: None

Patents/Licenses: None

Animal Models: In the second year, we have generated *Npm1*+/-*Arf*-/- and *Ddx5*+/-*Arf*-/- mice which will be free to any research that requests them.

Cell Lines: We have developed a unique primary mouse mammary epithelial cell (MMEC) line lacking the ARF tumor suppressor. These were established directly from *Arf* knockout mice on a pure C57Bl6 background. The *Arf*-null MMECs maintain a diploid phenotype and wild-type p53. These cells are spontaneously immortal and contain no artificial genes or plasmid constructs.

Funding Applied for: None

Employment Opportunities: Named Co-Director of the Breast Cancer Research Program, Siteman Cancer Center, Washington University School of Medicine, spring 2010.

CONCLUSION

We have shown that *Arf*-null mouse mammary epithelial cells (MMECs) contain unique polysome mRNA profiles. This might be due to the deregulation of Drosha protein expression which in turn is altering the production profile of miRNAs. These miRNAs would in turn function to alter the translational landscape of *Arf*-/- cells. Determining which miRNAs are important for the observed ARF phenotype will be critical in the next year.

We have made significant progress in our ability to identify signaling components that ultimately regulate ARF protein levels in response to classical oncogenic stimuli such as RasV12. We have now determined that crosstalk exists between the MAPK and mTOR pathways. This most likely results in enhanced ARF translation as we have been unable to detect any difference in either ARF mRNA or protein stability in the presence of RasV12. Understanding this crosstalk will be a major focus of the coming year.

We now understand the regulation of NPM translation. We have shown a clear mechanism of NPM translation by the 3'-UTR binding by FBP1. While the precise mechanism of FBP1 translational repression of NPM will be determined in the next year, we are confident that our unique reporter construct will provide critical insights.

Our unique efforts at understanding the functional interactions between ARF and NPM and p68DDX5 have yielded a plethora of data on the past two years. We now have mouse models implicating NPM and DDX5 as crucial downstream targets of ARF. In the next year, we will determine whether this functional interaction exists in MMECs and whether either protein presents itself as a clear target for therapeutic intervention in breast tumors lacking functional ARF.

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APPENDICES

None